Severely combined immunodeficient (SCID) mice resist infection with bovine spongiform encephalopathy

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Following combined intraperitoneal and intracerebral injection with bovine spongiform encephalopathy (BSE) cow brain homogenate, SCID mice show a resistance to infection in comparison with immunocompetent CB20 mice. BSE occurred in only five out of 22 challenged SCID mice, with a mean incubation period of 573 days, whereas all the CB20 mice developed the disease with a mean incubation period of 456 days. In contrast, previous studies have shown that intracerebral infection of SCID mice with a mouse-passaged scrapie strain, ME7, produces 100% incidence of disease but no replication of infectivity in spleen. The results with BSE suggest that there is little or no direct infection of the CNS in interspecies transmissions, but that processing or replication of infectivity in peripheral lymphoid tissues may facilitate subsequent spread of infection to the CNS.

Bovine spongiform encephalopathy (BSE) was first recognized in cattle in the United Kingdom in 1986, as a probable consequence of the consumption of scrapie-contaminated feed supplements (Wilesmith et al., 1991). Other species have also been affected, including exotic ruminants in zoological collections (Jeffrey & Wells, 1988) and domestic cats (Wyatt et al., 1990). In transmissions to mice from several cattle with BSE, a single major strain has been identified (Fraser et al., 1992; Bruce et al., 1994); this strain has also been isolated from cases of spongiform encephalopathies in cats and exotic ruminants, providing direct evidence for accidental spread of BSE to these species (Bruce et al., 1994). The recognition in 1996 of 10 cases of an unusual form of CJD (nvCJD; Will et al., 1996), occurring in relatively young people, raised the possibility that BSE has also spread to humans by dietary exposure.

A ‘species barrier’ is known to exist on cross-species infection with transmissible spongiform encephalopathies (TSEs). The effects of a change of species can include the selection of minor variant strains and a low efficiency of infection in establishing disease (Kimberlin et al., 1987, 1989). In addition, in BSE transmissions from cattle to mice, similar incubation periods are seen with intracerebral (i.c.) and peripheral routes of infection. In contrast, i.c. infection always produces disease more rapidly than peripheral infection when passages are carried out within a species. These findings suggest that a processing step in peripheral tissues, involving specific cells of the immune system, is required before infection can invade the CNS of the new species (Bruce et al., 1994).

Mature follicular dendritic cells (FDCs) in the spleen and lymph nodes are thought to play a key role in the pathogenesis of scrapie in mice (McBride et al., 1992). Genetically immunodeficient SCID mice lack functional B and T cells (Bosma et al., 1983) and, in addition, have immature and non-functional FDCs (Kapasi et al., 1993). SCID mice are also relatively resistant to peripheral challenge with mouse-adapted scrapie strains, but behave in the same way as immunocompetent CB20 mice following i.c. injection (Fraser et al., 1996). No replication is detected in the spleens of these mice, possibly as a result of the absence of replication sites associated with mature FDCs. In the present study, SCID mice were challenged with BSE from cattle to investigate the role of the lymphoreticular system (LRS) in the species barrier.

Three inbred mouse strains of the Sincα or Prn-pα genotype were used: SCID (CB17 scid/scid), CB20 (CB20+/−) immunocompetent controls and RIII (RIII/Fa/Dk). The RIII mouse strain was used as an incubation-period control on the source of BSE. Mice were maintained under specific-pathogen-free conditions with unlimited access to food and water. They were injected with a 5% unspun saline homogenate of BSE cow brain-stem by both the intraperitoneal (i.p.) and i.c. routes using a volume of 0·02 ml for the i.c. route and 0·1 ml for the i.p. route. The mice were coded and scored weekly for neurological signs to determine the clinical end-point and incubation period of the disease according to previously described criteria (Fraser & Dickinson, 1968). Brains from all mice were fixed in formol saline and coded haematoxylin- and eosin-stained paraffin sections examined to determine the presence of vacuolation diagnostic for TSEs (Fraser & Dick-
inson, 1968). Immunolabelling was carried out on selected brain sections to determine the presence of abnormal accumulations of PrP, which are also diagnostic for TSEs (Bruce et al., 1989). Brain sections were pretreated prior to immunostaining using the hydrated autoclaving method (Shin et al., 1991). The sections were then immunolabelled with one of two polyclonal antibodies to PrP: 1B3 (Farquhar et al., 1989) or 1A8 (Farquhar et al., 1994), using the standard avidin–biotin complex technique (Table 1).

At post-mortem, spleens from SCID mice were fixed in formol saline and haematoxylin- and eosin-stained sections examined to determine if ‘leakiness’, the production of lymphoid progenitor cells (Bosma et al., 1988), had occurred during the incubation period. In addition, sera from some animals were obtained to measure serum immunoglobulin using an ELISA method. Serum samples were compared with samples from immunocompetent animals and known SCID controls. Serum samples were coated onto flat-bottomed 96-well ELISA plates and incubated overnight at 4°C. Plates were washed and a rabbit anti-mouse IgG (HRP-conjugated, 1/1000 dilution; Dako) applied for 2 h at room temperature. O-Phenylenediamine dihydrochloride substrate (Sigma) was applied and incubated for 10 min at room temperature. Absorbance was measured at 490 nm using a microplate reader (Molecular Devices).

BSE was transmitted to RIII mice, producing an almost 100% incidence of disease and a mean incubation period of 349 days. This mean incubation period is consistent with those obtained in the same mouse strain in previous BSE studies (Fraser et al., 1992; Bruce et al., 1994), indicating a dose of approximately $10^2$ ID$_{50}$ units per mouse. Transmission of BSE to immunocompetent CB20 mice produced 100% incidence of disease and a mean incubation period of 456 days. Of the 36 SCID mice initially challenged with BSE, 14 were killed or died with intercurrent disease prior to the first positive CB20 case at 425 days p.i. and were excluded from the analysis. Of the 22 SCID mice surviving beyond 425 days, only five developed clinical disease, with a mean incubation period of 573 days. All five clinically positive SCID mice had vacuolar pathology and abnormal PrP staining in their brains. However, one of the 17 clinically negative SCID mice showed minimal abnormal PrP staining in focal clusters in the medulla and a PrP-positive plaque in the thalamus, in the absence of vacuolar pathology. This mouse was killed with an intercurrent illness 468 days p.i. The remaining clinically negative SCID mice had no vacuolar pathology and no abnormal PrP staining.

Spleens from the five SCID mice which developed BSE were examined histologically to determine if the SCID phenotype had been retained during the incubation period (Table 2). Of the five, one was assessed as having a ‘leaky’ phenotype by examination of haematoxylin- and eosin-stained spleen sections, which revealed that the T and B cell areas of the white pulp, normally very small in immunodeficient spleens, were well developed. This SCID mouse had the shortest incubation period of the five, at 467 days, close to the mean incubation period of the CB20 mouse. Of the four remaining positive SCID mice, three had no distinguishable lymphoid structure in their spleens, consistent with an immunodeficient phenotype. Spleen was not available for analysis from the fourth mouse, but in this case the lack of serum immunoglobulin indicated an immunodeficient phenotype had been retained.

In this study BSE has been shown to transmit less efficiently

### Table 1. Incidence and incubation periods of BSE (mean ± standard error) in RIII, CB20 and SCID mice following combined i.p. and i.c. injection with a 5% BSE brain homogenate

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Incidence</th>
<th>Incubation period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIII</td>
<td>20/21*</td>
<td>349 ± 13</td>
</tr>
<tr>
<td>CB20</td>
<td>7/7</td>
<td>456 ± 12</td>
</tr>
<tr>
<td>SCID</td>
<td>5/22†</td>
<td>573 ± 37‡</td>
</tr>
</tbody>
</table>

* One mouse died 398 days post-injection and showed negative vacuolar pathology.
† 36 SCID mice were initially injected with BSE. 14 mice were killed intercurrently, before the first positive BSE case in the CB20 mice (425 days p.i.), and are excluded from the denominator.
‡ Significant difference between mean incubation periods of SCID and CB20 mice; two-tailed t-test, $P = 0.0316$. Excluding the ‘leaky’ SCID from the analysis gives a mean incubation period ± SE of 599 ± 34; two-tailed t-test, $P = 0.0178$.

### Table 2. Determination of SCID phenotype using histological analysis of spleen or serum immunoglobulin (Ig) measurement

<table>
<thead>
<tr>
<th>Spleen number</th>
<th>Incubation period (days)</th>
<th>Phenotype assessed by spleen morphology* or ELISA to determine serum Ig†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>651</td>
<td>SCID</td>
</tr>
<tr>
<td>2</td>
<td>572</td>
<td>SCID‡</td>
</tr>
<tr>
<td>3</td>
<td>658</td>
<td>SCID</td>
</tr>
<tr>
<td>4</td>
<td>517</td>
<td>SCID</td>
</tr>
<tr>
<td>5</td>
<td>467</td>
<td>Leaky SCID</td>
</tr>
</tbody>
</table>

* Spleen morphology was compared with known SCID spleen controls.
† Serum was only available from spleens 1 and 2. An ELISA method using absorbance readings obtained at 490 nm was used to determine SCID phenotype. Serum from immunocompetent and non-leaky SCID mice were used as controls.
‡ This spleen was not available for analysis.
to SCID mice than to immunocompetent control mice, producing prolonged incubation periods in the few SCID mice that developed the disease. This implies that the i.c. component of the BSE challenge was inefficient in establishing infection directly into the brains of these mice. This does not occur when SCID mice are challenged with an equivalent dose of a mouse-passaged scrapie strain, where injection directly into the brain always initiates infection locally (Fraser et al., 1996). These results also support the hypothesis that routing of infection through an intact LRS is an important prelude to neuroinvasion in cross-species transmissions. However, the successful transmission of BSE to a few SCID mice that retained their immunodeficient phenotype may suggest other less efficient mechanisms of pathogenesis that do not involve the LRS, such as direct infection of the CNS or peripheral nerves.

Although the molecular nature of TSE agents is still uncertain, it is likely that they are composed partially or wholly of abnormal isoforms of PrP (Dickinson & Outram, 1988; Prusiner, 1982). It has been suggested that the species barrier is a consequence of incompatibility between PrPs of the ‘donor’ and ‘recipient’ species (Prusiner et al., 1990). If this is the case, it is possible that this incompatibility prevents direct infection of the CNS, but that this barrier may be overcome by infection of less fastidious cells outside the CNS. An obvious candidate cell is the FDC, which is immature in the SCID mouse (Kapasi et al., 1993). Once infection has been established extraneurally, infectivity associated with PrP of the new host may then spread to the CNS by the usual mechanisms. However, factors other than a simple incompatibility between PrPs from different species are also likely to contribute to the species barrier (Bruce et al., 1994). Whatever the basis of our results, the present study highlights the importance of investigating TSE pathogenesis following more ‘natural’ peripheral routes of infection. Some previous studies of interspecies transmission, using only i.c. challenge, have artificially exaggerated the species barrier. More realistic indicators of the species barrier are the relative incubation periods and efficiencies of infection using peripheral routes of challenge on both primary transmission and subsequent passage within the new species.

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References


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